Cancer battlefield: six characters in search of an author

"…single-target therapy is fading in favor of a multitarget approach. Inhibiting both cancer cells and the cells in the supporting tumor stroma, as well as restoring and maintaining T-cell function, will probably provide a path for success for adoptive T-cell therapy in the fight against tumors."

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The great promise of adoptive T-cell therapy for cancer is that of a highly specific and less toxic strategy for killing tumor cells while offering continual and long-term protection against resurgence of tumors. Although theoretically possible, this promise has yet to be achieved in practice. Encouraging results initially came from studies in which tumor-infiltrating T cells were isolated and then expanded and reinvigorated *in vitro* before being infused back into patients [1,2]. While promising, this approach, however, requires both access to tumor biopsies, which are not always available, and the ability to expand T cells from the tumor to sufficient numbers for infusion, which is not always possible. Thus, to extend T-cell therapies to more patients, much effort has been put into developing methods for the *ex vivo* expansion of tumor-specific T cells from peripheral blood, and while successful for virus-associated tumors, the extension to other human malignancies expressing nonviral tumor associated antigens remains challenging due to the low frequency and avidity of tumor-specific T cell receptors (TCRs) on patient-derived T cells [3]. To overcome the inefficient generation of tumor-associated antigen-specific T cells, genetic engineering of lymphocytes with αβ-T-cell receptor chains, obtained from T cells with high avidity/affinity TCRs, or chimeric antigen receptors, directed against antigens expressed on the surface of tumor cells, have been developed and are now in clinical trials [4,5]. These efforts have been efficient at providing relatively easy means to broaden the antigen recognition repertoire and to generate large numbers of tumor-specific T cells *ex vivo* that can then be infused into patients to re-establish the balance between T cells and tumors. However, even the ability to infuse large numbers of tumor-specific T cells has provided limited advancement in the overall fight against tumors.

The reasons for these unmet expectations stem from limiting our attention to only the main characters, the tumor cells (and which antigens they express) and tumor-specific T cells (and which antigens they recognize), when designing T-cell therapies for cancer. T cells can eliminate tumors only if they efficiently reach and penetrate tumor sites, as well as maintain their capacity to proliferate and function within the tumor. However, tumors do not make this easy and they limit T-cell functions by overexpressing antiapoptotic factors, such as MCL-1, and a number of inhibitory ligands and factors, such as PDL-1, indoleamine 2,3-dioxygenase (IDO), IL-10, TGF-β and VEGF [6]. Furthermore, tumor cells secrete chemokines, including CCL17, CCL22 and SDF-1, which specifically recruit populations of immune cells to promote tumor growth not only by supplying growth factors and several nutrients but also by establishing a multitude of immune evasion mechanisms. These cells, which make up the supporting cast, include regulatory T cells (Tregs), myeloid cells (dendritic cells [DCs] and macrophages), fibroblasts and endothelial cells. Their critical contribution to tumor pathogenesis has only recently begun to be recognized.

Tregs represent a subset of CD4+ T cells characterized by the constitutive expression of the high-affinity IL-2 receptor (CD25) and the transcription factor FOXP3. These cells are crucial mediators of peripheral tolerance and immune suppression and are often found in abundance in the tumor microenvironment, with increased numbers often predicting poor survival [7]. Immune suppression mediated by Tregs relies on multiple mechanisms, including secretion of

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cytokines, such as IL-10 and TGF-β, which not only suppress effector T cells but can also polarize DCs to tolerogenic phenotypes; induction of target cell cytolysis through a variety of mediators such as Trail, galectin and granzyme B; starvation of essential stimulatory cytokines such as IL-2; and finally by inducing DCs to express IDO, which diminishes the level of the essential amino acid, tryptophan, and generates suppressive metabolites.

The crucial role of myeloid cells (DCs, macrophages and myeloid-derived suppressor cells) in promoting tumor angiogenesis, cell invasion and metastasis has only recently become evident. Many soluble factors (e.g., VEGF) present in the tumor microenvironment impair DCs maturation and function, so that the DCs in tumor-bearing hosts or found in the tumor environment are often immature and tolerize rather than stimulate immune responses. Macrophages in tumors generally have reduced capacity for antigen presentation and usually suppress antitumor T-cell responses through secretion of IL-10 and TGF-β as well as chemokines, such as CCL12 and CCL22, responsible for the recruitment of Th2-polarized immune cells and activation of Tregs, a phenotype known as M2. M2 macrophages also produce proangiogenic factors and angiogenic modulating enzymes that facilitate tumor metastasis [8]. Myeloid-derived suppressor cells, a heterogeneous population of immature myeloid cells, similarly suppress T-cell responses by expressing arginase, which depletes the amino acid, arginine and iNOS, which produces nitric oxide, and by increased production of reactive oxygen species [9].

Cancer-associated fibroblasts/mesenchymal stromal cells contribute to tumor proliferation and metastasis through the secretion of multiple growth factors, cytokines and chemokines, such as SDF-1, which directly recruit endothelial and immune cells to the tumor site. Cancer-associated fibroblasts/mesenchymal cells can also directly suppress antitumor immune responses through the secretion of inhibitory factors, such as TGF-β and PGE2, expression of regulatory enzymes, such as IDO, and expression of coinhibitory ligands, such as PDL-1 [10].

The recruitment and proliferation of endothelial cells promotes tumor angiogenesis, which is crucial in the development and growth of tumors and for their hematogenous dissemination. Blood vessel development is critical for the supply of essential nutrients and growth factors and for the removal of waste from tumors. The tumor endothelium also contributes to the inhibitory microenvironment by producing an array of inhibitory cytokines and molecules and by providing an immunosuppressive barrier, limiting access of tumor-specific T cells [11].

Altogether, the multiple interactions between these various supporting cells and tumor cells establishes a complex microenvironment that is being increasingly recognized as integral to the outcome of tumor therapies, including T-cell therapies. We can reinvigorate tumor-specific T cells *in vitro* but, upon infusion, they will re-encounter the immune suppressive tumor microenvironment. Although gene modifications of adoptively transferred T cells can help overcome one or a few of these immune evasion mechanisms at a time, the most successful T-cell therapies will probably involve strategies that not only target tumors but also address and break multiple elements in the tumor microenvironment. Support for a multitargeted approach comes from the impressive results obtained with T-cell therapies in patients that received preconditioning cytotoxic lymphodepleting chemotherapy [2,12] to remove Tregs and cells that act as cytokine sinks, which increases the availability of homeostatic cytokines that promote the expansion and functionality of the infused T cells. Preconditioning regimens are also useful as they are thought to have effects on the tumor vasculature and influence T-cell trafficking by increasing the expression of adhesion molecules [13]. Although cytotoxic lymphodepleting regimens target multiple aspects of the tumor microenvironment, they have their own set of limitations and significant adverse side effects that prevents their use in many patients.

Ideally, T-cell therapies will deliver on the promise of reduced overall toxicity. Now the challenge becomes identifying and developing minimally toxic strategies that can substitute for cytotoxic lymphodepleting chemotherapy in targeting multiple suppressive elements in the tumor microenvironment. Several approaches are currently being evaluated by various groups. These include the generation of T cells that specifically target stromal elements [14] or deliver powerful Th1-inducing cytokines, such as IL-12, to reprogram cells in the tumor microenvironment [14–16]. Each of these approaches could be combined with other genetic modifications of T cells that provide resistance to immune suppression, such as the dominant-negative TGF-βRII [17], or enhanced function, such as incorporation of additional costimulatory domains in chimeric antigen receptors (third generation) or provision of cytokines [4]. In addition, upon ensuring lack of interference with T-cell function, genetically

modified T cells could be combined with small-molecule inhibitors that can downregulate multiple tumor immune evasion mechanisms and inhibit angiogenesis [18]. To this end, a promising combination may be with mTOR inhibitors, provided a favorable schedule for dosing of this small-molecule inhibitor and T cells is designed or upon genetic modification of the T cells to specifically protect them from the negative effects of the drug on lymphocytes [19,20].

In conclusion, single-target therapy is fading in favor of a multitarget approach. Inhibiting both cancer cells and the cells in the supporting tumor stroma as well as restoring and maintaining T-cell function will probably provide a path for success for adoptive T-cell therapy in the fight against tumors.

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